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Activatable albumin-photosensitizer nanoassemblies for triple-modal imaging and thermal-modulated photodynamic therapy of cancer

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ABSTRACT

Photodynamic therapy (PDT) is a noninvasive and effective approach for cancer treatment. The main bottlenecks of clinical PDT are poor selectivity of photosensitizer and inadequate oxygen supply resulting in serious side effects and low therapeutic efficiency. Herein, a thermal-modulated reactive oxygen species (ROS) strategy using activatable human serum albumin-chlorin e6 nanoassemblies (HSA-Ce6 NAs) for promoting PDT against cancer is developed. Through intermolecular disulfide bond crosslinking and hydrophobic interaction, Ce6 photosensitizer is effectively loaded into the HSA NAs, and the obtained HSA-Ce6 NAs exhibit excellent reduction response, as well as enhanced tumor accumulation and retention. By the precision control of the overall body temperature instead of local tumor temperature increasing from 37 °C to 43 °C, the photosensitization reaction rate of HSA-Ce6 NAs increases 20%, and the oxygen saturation of tumor tissue raise 52%, significantly enhancing the generation of ROS for promoting PDT. Meanwhile, the intrinsic fluorescence and photoacoustic properties, and the chelating characteristic of porphyrin ring can endow the HSA-Ce6 NAs with fluorescence, photoacoustic and magnetic resonance triple-modal imaging functions. Upon irradiation of low-energy near-infrared laser, the tumors are completely suppressed without tumor recurrence and therapy-induced side effects. The robust thermal-modulated ROS strategy combined with albumin-based activatable nanophotosensitizer is highly potential for multi-modal imaging-guided PDT and clinical translation.

1. Introduction

Photodynamic therapy (PDT) is a clinically approved therapeutic modality for the treatment of various diseases including cancers [1]. It predominantly utilizes the light triggering the photosensitizer convert tissue oxygen (O_2) to highly reactive oxygen species (ROS) for destroying near cancer cells [2,3]. This type of light-triggered and O_2-dependent therapeutic method has remarkably improved selectivity and fewer side effects as compared to traditional anticancer therapies [4]. However, inadequate O_2 supply in solid tumors (hypoxia) led to the low ROS generation efficiency [5,6]. Photosensitizer-mediated depletion of O_2 exacerbates tumor hypoxia, leading to lower efficiency of PDT against cancer [7,8]. Moreover, currently available photosensitizers are mostly nonspecifically activated and exhibit a low tumor selectivity, which causes treatment-related toxicity and side effects on adjacent normal tissue and blood cells [9,10].

To overcome the limitations, efforts have been made to improve the ROS generation efficiency and enhance the tumor selectivity of photosensitizers. One strategy is the directly increasing of O_2 concentration in tumor tissue with intelligent nanomaterials that can "fabricate" O_2 directly in tumor microenvironment. For instance, Guo et al. reported H_2O_2-activatable and O_2-evolving nanoparticles for overcoming tumor hypoxia and promoting the generation of

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ROS [11]. Shi et al. explored the excessive amounts of H_2O_2 in tumor tissue could be decomposed by MnO_2 nanosheets for circumventing the tumor hypoxia [12]. Another approach is the combination PDT and other therapeutic modalities for improving treatment efficiency [13,14]. For example, photothermal therapy (PTT) could be coordinated with PDT to promote the delivery of photosensitizer and enhance the PDT efficacy against cancer cells [15]. Moreover, recent study indicated that single continuous wave laser could induce simultaneously PTT (above 43 °C) and PDT for synergistic cancer phototherapy, which could further simplify the treatment process [16,17]. However, these methods remained to integrate different functionalities by accumulating various materials into a single and efficient nano-system, which contained multiple steps and complexity during the synthetic processes, resulting in a difficult optimization [18]. Moreover, local thermal effects induced by physical or chemical approaches almost hardly increased the O_2 supply in tumor tissue, resulting in the low ROS generation efficiency [16].

Herein, we develop a thermal-modulated ROS strategy for increasing O_2 supply in tumor tissue and improving the PDT efficiency. This design concept is inspired by the "Hot Spring" bath, which emphasizes the key role of appropriately raising the body temperature for accelerating photosensitive reaction rate and increasing the O_2 levels in tumor tissue. From this perspective, this proposed strategy differs from local thermotherapy induced by laser or microwave for enhancing PDT [10,19]. Chlorin e6 (Ce6), an intrinsic theranostic photosensitizer with a high sensitizing efficacy, near-infrared fluorescence (FL), photoacoustic (PA) imaging, and chelating manganese ion (Mn^{2+}) features [20–23], can be effectively loaded into the human serum albumin nano-assemblies (HSA NAs) via hydrophobic interaction and intermolecular disulfide bond cross-linking of HSA protein (Fig. 1a). The HSA-Ce6 NAs exhibit unique characteristics as following: (i) facile assembly: intermolecular disulfide bond cross-linking of HSA protein without introducing exogenous cross-linking or toxic agents. (ii) Excellent tumor selectivity: passive and active tumor-targeting mediated by enhanced permeability and retention effect and receptor-mediated transcytosis [24–27]. (iii) activable theranostics: reduction-responsive fluorescence imaging, PA imaging and magnetic resonance imaging (MRI)-guided highly efficient PDT with low side effects.

2. Materials and methods

2.1. Materials

HSA was obtained from Beijing Biosynthesis Biotechnology CO., LTD. Ce6, GSH, propidium iodide (PI), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 4,6-diamidino-2-phenylindole (DAPI) were purchased from Sigma Aldrich. Calcein-AM and Alexa Fluor 488 Annexin V/Propidium Iodide (PI) Cell Apoptosis Kit were obtained from Invitrogen (USA). Phosphate-buffered saline (PBS, pH 7.4), fetal bovine serum (FBS), RMPI 1640, trypsin-EDTA and penicillin-streptomycin were purchased from Gibco Life Technologies (AG, Switzerland). Ethanol was obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). All other chemicals used in this study were of analytical reagent grade and used without further purification. Ultrapure water (18.25 MΩ cm, 25 °C) was used to prepare all solutions. BALB/c athymic nude mice and BALB/c mice were maintained under aseptic conditions in a small animal isolator. All food, water, bedding and cages were autoclaved before use.

2.2. Preparation and characterization of HSA-Ce6 NAs

80 mg HSA was firstly dissolved in 2 mL deionized water with 50 mM GSH at 37 °C for 1 h. Then, 8 mL of the ethanol (containing 2 mg/mL Ce6) was added into the solution to precipitate the HSA-Ce6 NAs. The suspension was kept under stirring at 37 °C for 10 min. After that, the suspension was dialyzed (membrane cutoff MW: 12 K–14 K Da) in deionized water for 12 h at 4 °C. During the dialysis process, ethanol molecules could permeate into the water across the dialysis membrane. Therefore, dialysis solution was gradually changed from pure water to the water-ethanol mixed solution, which could enhance the water solubility of Ce6 and allow to removing the unbound fraction of Ce6. To determine Ce6 loading efficiency, the HSA-Ce6 NAs solution was diluted in 5 mL of ethyl acetate/ethanol (9:1, v/v) and sonicated for 30 min to extract Ce6 completely. Ce6 levels were determined by UV–Vis absorption spectra. The loading efficiency of Ce6 was defined as Ce6 content (%, w/w) = (Ce6 weight in NAs/total NAs weight) × 100. All the measurements were performed in triplicate. For chelating with Mn^{2+}, the prepared HSA-Ce6 NAs were mixed with $MnCl_2$ solution at a molar ratio of 2:1 for 2 h. Excess $MnCl_2$ was removed using a dialysis process (MWCO = 100 kDa). The concentration of Ce6 molecular and Mn^{2+} in the HSA-Ce6-Mn^{2+} NAs were determined by UV–vis absorption spectrum and ICP-AES, respectively.

2.3. In vitro Ce6 release of HSA-Ce6 NAs

To determine Ce6 release profile, 2 mL HSA-Ce6 NAs solution was closed into a dialysis bag (membrane cutoff MW: 10 K Dalton). Then the bag was immersed into 50 mL releasing solution (PBS, 0.01 mol/L, pH = 7.4 and 37 °C) with different GSH concentration. At certain time points, the release solution was withdrawn for absorption spectrophotometer analysis, and same volume of releasing solution was added.

2.4. Detection of ROS in HSA-Ce6 NAs

2′,7′-dichlorofluorescin diacetate, which was highly sensitive to ROS, was employed here during the detection process. Different samples were mixed with 2.5 μmol 2′,7′-dichlorofluorescin diacetate, and then irradiated by a 660 nm laser (200 mW/cm^2) for different periods of time. The generation of ROS was determined by measuring enhanced fluorescence of DCF ($\lambda_{excitation}$ = 504 nm and $\lambda_{emssion}$ = 529 nm).

2.5. Cell culture

The 4T1 mouse breast cancer cells were cultured in RMPI 1640 supplemented with 10% (v/v) fetal bovine serum, 1% (v/v) penicillin, and 1% (v/v) streptomycin. Cells were incubated in a humidified incubator at 37 °C with 5% CO_2.

2.6. Cell viability assays

4T1 cells were cultured in standard cell media. Cells were first seeded into 96 well plates then incubated with free Ce6 and HSA-Ce6 NAs with different concentrations for 24 h. The standard MTT assay was used to determine relative cell viabilities.

2.7. In vitro cellular uptake

For *in vitro* studies, 1×10^5 4T1 cells were seeded on a glass-bottomed culture dish, respectively. After 24 h, HSA-Ce6 NAs or free Ce6 was incubated with 4T1 cells for 3 h at 37 °C or 43 °C, respectively. In order to study the reduction-responsive of HSA-Ce6